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**I-USNIC ACID AND OBSERVATIONS ON ITS
EFFECT IN HUMAN TUBERCULOSIS**

BY

JORMA PÄTIÄLÄ and RISTO PÄTIÄLÄ

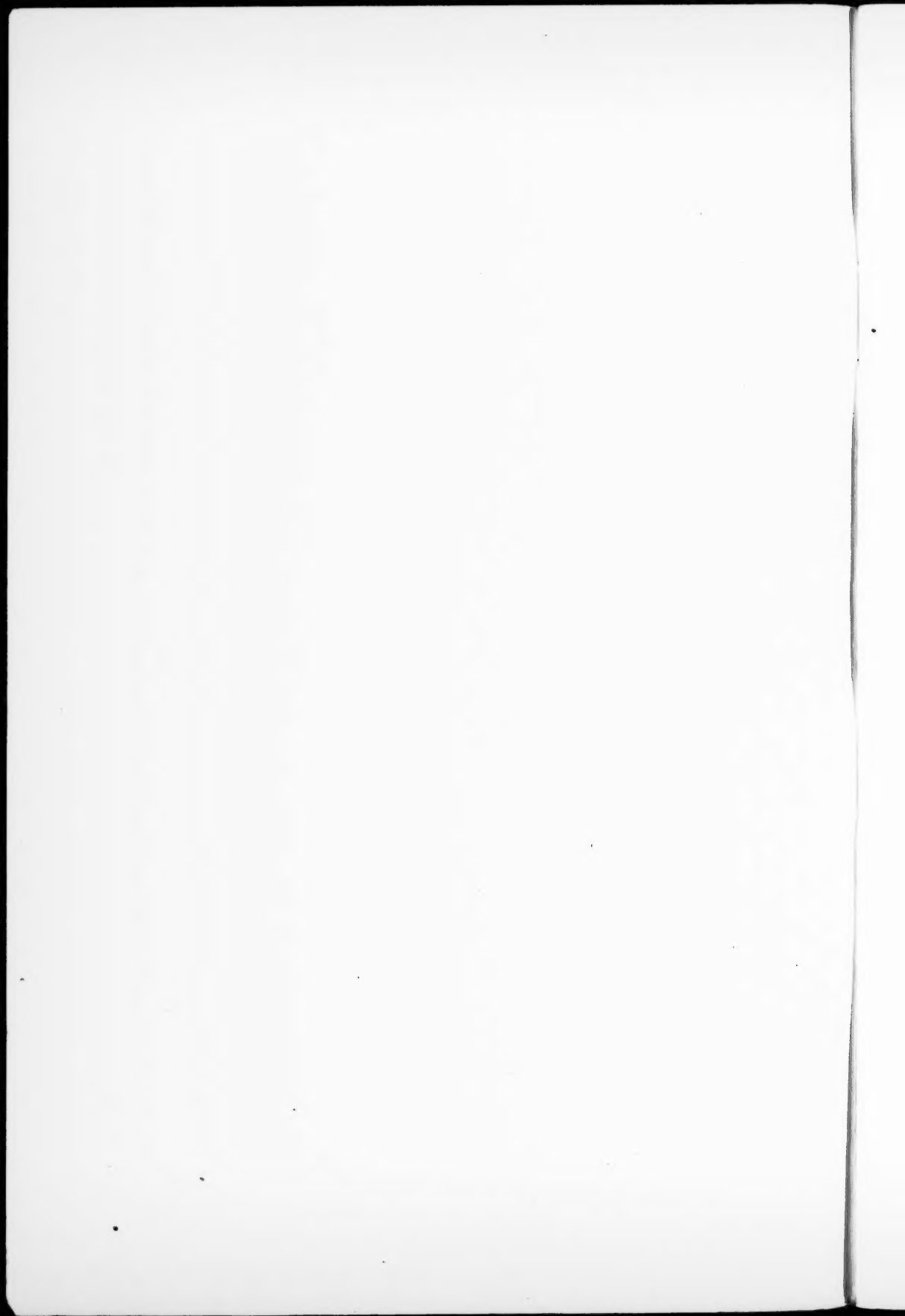
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INTRODUCTION

Since time immemorial, lichen has been used as a medicinal plant by the country people. Lichen preparations have also been included in official pharmacopoeias, for instance as an antitussive, and still to-day Iceland moss, *Cetraria islandica*, is listed in a few pharmacopoeias. After the discovery of antibiotic and bactericidal substances in many of the old popular medicines, scientific interest in lichens also increased. The d- and l-usnic acids in particular, on which the antibiotic activity of many lichens is based, have been a subject of study. Especially after the discovery that usnic acid is effective also against species of *Mycobacterium tuberculosis* both *in vitro* (Stoll *et al.* 1947, Marshak 1947, Marshak, Barry *et al.* 1947, Shibata *et al.* 1948) and *in vivo* (Marshak 1947, Pātiälä, *et al.* 1948), continued investigation and interest have been directed toward this substance.

ISOLATION OF USNIC ACID

Usnic acid was isolated from beard moss by Rochleder and Heldt already in 1843. This acid has later been found to be present especially in various lichen species of the genus *Cladoniae*. When in 1947, without knowledge of the recent research work in this field in other countries, our studies of usnic acid were started in Finland, we employed for preparation of the acid the reindeer moss, *Cladonia alpestris*, a lichen of the above mentioned genus growing extensively

in Finland. The first batch of acid was prepared from dry, powdered lichen by extraction with boiling acetone. Most of the acid was crystallised from the extract in the form of needlelike, bright yellow crystals in 24 hours. The acid thus obtained was re-purified by crystallisation. The yield of acid from the plant was 1.5 per cent by weight. The needlelike crystals had a melting point of about 202° C and a specific weight of about 1.3. The acid and its sodium salt were at first tasteless to the tongue but two or three minutes later had a very bitter taste. In the course of two years 30 species of lichen native to Finland were studied for the determination of the best raw material for usnic acid. However, reindeer moss continued to be the best in view of its high usnic acid content, combined with an extensive habitat (Meretoja 1952). By combination of ether-alcohol and water-alcohol extractions and by crystallisation with chloroform, Meretoja was able to obtain from the dry weight of the lichen 1 per cent of acid with a melting point of 211° C and a specific rotation of $(\alpha)_{\text{D}}^{15} = -496^\circ$.

CERTAIN CHEMICAL AND PHYSICAL PROPERTIES OF USNIC ACID

Although usnic acid has been known for over 100 years, some degree of variance still exists in the data published by various investigators on its chemical and physical properties. Thus for instance Widman (1900) reported a melting point of 203° C for the d- and l-forms, Asahina *et al.* (1934) 202° C and Shibata *et al.* (1948) 199° C for the d-form, and Zopf (1907) 196° C, Shibata *et al.* 201° C, Siintola, Pātiälä *et al.* (1948) 202° C, Vartia (1950) 197—198° C, Laakso *et al.* (1952) 202—204° C and Meretoja (1952), as said above, 211° C for the l-form. In determinations of the melting point by the capillary method, which caused decomposition of the substance, Meretoja obtained 198° C as the melting point of the decomposition product. Although the divergent specifications may to some extent be ascribed to differences in the methods of determination, it may rather be assumed that the tendency of usnic acid to decompose and the presence of decomposition products or of impurities have influenced the results obtained by the various workers.

Regarding the solubility of usnic acid it may be mentioned that the acid is practically insoluble in water or glycerin and poorly

soluble in methyl, ethyl and isopropyl alcohol and in petroleum ether and petrol. On heating it is readily soluble in, *e.g.*, camphor, phenol, liquid paraffin, oil of turpentine, aniline, benzol, xylol, toluol, chloroform, trichlorethylene, tetrachlormethane, dioxane, glycol, propylene glycol, and polyethylene glycol. It also dissolves and forms salts in sodium hydroxide and in a solution of sodium bicarbonate and it is likewise soluble in concentrated sulphuric acid. When the solution, which has an intense yellow colour, is allowed to stand on a watch glass for a few hours, a blue film is formed on the surface and the colour of the underlying liquid becomes lighter. This reaction is influenced by the atmospheric moisture, for the colour of the acid treated similarly in an exsiccator remains unaltered for several days. The yellow colour of usnic acid is, as said, very intense, and a solution made with glycol in the ratio of 1: 3,000 is still clearly yellowish.

Usnic acid can also be isolated from the lichen by microsublimation, the acid being at first isolated in oily droplets, which then form yellow crystals (Siintola *et al.* 1948, Pātiälä *et al.* 1950).

As said, usnic acid is poorly soluble in water. Heilala *et al.* (1949) found the solubility in water to be less than 1: 5,000,000, whereas that of its sodium salt was about 1: 600. In his study of the solubility of l-usnic acid in certain solvents (17° C) Meretoja reached the following results:

Solvent	Solubility of l-Usnic Acid	
	mg/ltr	w/w
Water	2.81	1: 356,000
Ethanol	44.0	1: 18,000
Hexane	116.1	1: 5,700
Ether	1,107.0	1: 650

Siintola, Pātiälä *et al.* (1948) carried out determinations of the absorption spectrum (A) of usnic acid in the Beckmann quartz spectrophotometre in the range of ultra violet rays and in the Pulfrich photometre in the range of visible rays. Chloroform was used as solvent, the strength of the usnic acid was 0.0166 mg/ml, and the depth of the fluid layer 1 cm. The following absorption values were obtained at the wavelengths indicated (m μ):

m μ	420	410	400	390	380	370	360	350
A	0.032	0.060	0.097	0.135	0.171	0.204	0.233	0.269

$m\mu$	345	340	330	320	310	300	295	290
A	0.290	0.315	0.352	0.405	0.497	0.687	0.860	1.052
$m\mu$	285	284	283	282	281	280	275	270
A	1.166	1.167	1.163	1.153	1.137	1.125	0.995	0.848
$m\mu$	265	260	255	253				
A	0.712	0.605	0.575	0.554				

The third decimal was not certain between wavelengths 290 and 280 $m\mu$. The shape of the curve is seen in fig. 1.

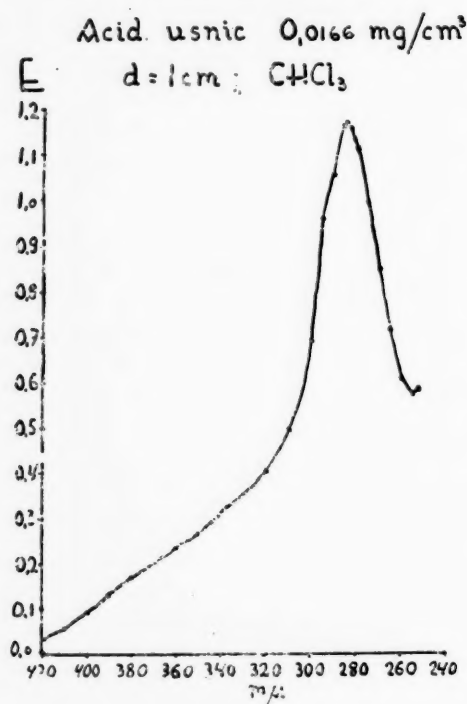


Fig. 1.

In the Pulfrich photometre the following percentages were obtained for the optical transparency (CHCl₃ solution, 5 mg/cu cm, d = 1 cm):

$m\mu$	435	463	494	530	572	617	666	728	750
T %	7.2	58.6	77.8	79.4	81.1	80.6	81.1	81.1	82.6

Fig. 2 shows the curve obtained for the Pulfrich values.

A few crystals of usnic acid were dissolved in ethylene glycol or in propylene glycol. In both cases a yellow solution was obtained,

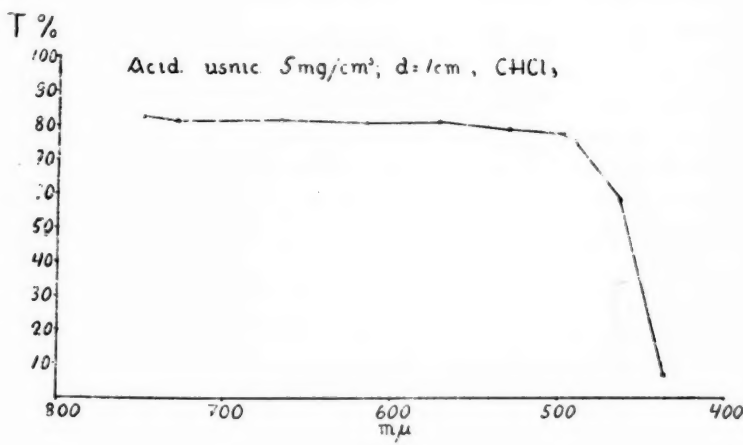
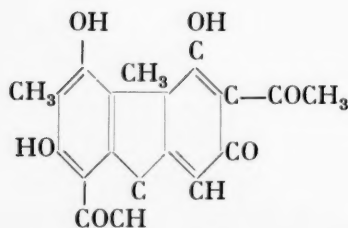


Fig. 2.

and on addition of a small amount of the alcoholic solution of the above mentioned reagent the mixture turned green. This colour persisted unchanged for some hours. The intensity of the colour was found to depend on the concentration of the usnic acid solution; in a dilution of 1:4,000 the colour was still visible. By the use of suitable amounts of reagent and acid the concentration of the usnic acid solution could be determined in the Duboscq colorimeter, using as standard a pure solution of usnic acid of known concentration. It was possible to determine in this manner the concentration of acid in high dilutions (1:1,000; 1:4,000).

The same uncertainty as in the melting point of usnic acid is also seen in the values reported in the literature for its specific optical rotation. That of the d-form has been reported by Widman as $+492$ – 495° , by Schöpf *et al.* (1927) as $+492^\circ$, by Asahina *et al.* as $+490$ – 500° and by Shibata *et al.* as $+469^\circ$, and that of the l-form as -480° by the last mentioned workers. Siintola, Pätälä *et al.* (1948) obtained a specific rotation of l-usnic acid from *Cladonia alpestris* of $(\alpha)_D^{20} = -452^\circ$, Vartia -492° , and Meretoja -496° .

The molecular formula of usnic acid has for a long time been known to be $C_{18}H_{16}O_7$ and the molecular weight 344.1. Unanimity has also been reached regarding its structural formula. The error in the structural formula presented by Widman in 1900 was proved by Schöpf *et al.* and later, among other workers, by Curd *et al.*



(1933). The structural formula for usnic acid has been presented in this form by Florey *et al.* in their monograph »Antibiotics» (1953).

Several studies have also been carried out on the decomposition of usnic acid. Among its decomposition products may be mentioned decarbousnic acid ($C_{17}H_{16}O_6$), usnetinic acid ($C_{14}H_{14}O_6$), usneol ($C_{11}H_{12}O_3$) and pyrousnic acid ($C_{12}H_{12}O_5$).

The toxicity of usnic acid was studied by Mikoshiba (1936), who observed an action on smooth muscles similar to that of papaverine, although not as toxic. The lethal dose for the mouse was found to be 7.0 mg/10 g subcutaneously and 0.25 mg/10 g intravenously. Experiments on the toxic properties of usnic acid have also been made by other investigators and will be referred to later in connection with the experiments in this report.

EXPERIMENTS IN VITRO

Stoll *et al.*, in 1947, found that the antibiotic action of lichens is in many cases due to d- or l-usnic acid. This action is very marked against the causative agent of tuberculosis. The growth of some *Mycobacterium tuberculosis* species was completely inhibited by usnic acid in concentrations of 1: 500,000—1: 800,000. Marshak isolated in 1947 from the California Spanish moss (*Ramalina reticulata*) a yellow crystalline substance, which he later (1947) identified as d-usnic acid. Inhibition of *Mycobacterium tuberculosis* species was complete in a concentration of 1: 50,000 and was still noticeable in 1: 2,000,000. Bustinza and Caballero (1948) observed that d-usnic acid inhibited the growth of *Mycobacterium phlei* at a concentration of 1: 100,000 and of *Mycobacterium tuberculosis avium* completely at 1: 100,000 and partially at 1: 200,000. They also made the interesting observation that an aqueous solution of the sodium salt of usnic acid added to streptomycin hydrochloride was more effective against species of *Mycobacterium* than the sodium

salt or the streptomycin alone, and that it also had an inhibitive action on *E. coli*. Shibata *et al.* reported that usnic acid and its sodium salt were effective against *Myc. tub. avium* in the concentration of 1: 160,000. In later studies on the action of usnic acid and its derivatives, Marshak *et al.* (1949) found that among 30 derivatives only monoacetylusnic acid in a concentration of 1: 100,000 had the inhibitory action of usnic acid against *Myc. tub. hominis*. Siintola, Pātiälä *et al.* (1948) and Vartia (1949) obtained corresponding results with usnic acid in a concentration of 1: 60,000 and Klosa (1949) in 1: 1,000,000. Laakso and Brander (1952) made an attempt to compound para-aminosalicylic acid (PAS) and l-usnic acid, to which an equal part of methylglycosamide had been added to improve the solubility. They tested the action of this compound against both pathogenic and saprophytic acid-fast bacilli and compared the results with those obtained with PAS and usnic acid alone. The compound, however, had a definitely weaker action than PAS when used alone but a more potent effect than l-usnic acid alone. Thus PAS dominated in the PAS-l-usnic acid compound. The tuberculostatic concentration of l-usnic acid was determined by them to be 14.1—28.2 γ /ml, which corresponded to the values earlier obtained in Finland (Pātiälä, Vartia). On the other hand, in the case of saprophytic acid-fast bacilli (*Myc. smegmatis*, Rabinovitch R., *Myc. enteritidis*, Thimoteo, Battaglini, Vallée gelb) PAS used alone was least effective, as more than 1024 γ /ml was required for inhibition of bacterial growth, whereas 64 γ /ml of l-usnic acid was sufficient. The PAS-usnic acid compound was approximately similar in effect. It therefore seems evident that the compounding of PAS with usnic acid does not in any way alter the effect of l-usnic acid. It has recently been demonstrated *in vitro* by Virtanen *et al.* (1954) that l-usnic acid derivatives of p-acetylaminobenzaldehyde thiosemicarbazide (Conteben) (1: 20 million) and sulphaguanidine (1: 5 million) are very potent inhibitors of *Myc. tuberculosis*.

ANIMAL EXPERIMENTS

Very few experiments have been reported up to the present on the effect of usnic acid *in vivo*. In his first study in 1947, Marshak observed that the yellow crystalline substance isolated by him from *Ramalina reticulata* and administered by subcutaneous injection appeared to retard the progress of induced human tuberculosis

in guinea pigs. It was possible to administer a daily dose of 10—30 mg for a period of 3 weeks without obvious toxic effect. After treatment with subcutaneous injections the mortality during 32 days among guinea pigs infected with strain H₃₇RV was 30—40 per cent of the mortality of untreated guinea pigs, which was 75 per cent. Visceral changes were slight in the treated animals, but very clear tuberculous changes were seen in the viscera of the untreated animals. In continued experiments reported in 1950, Marshak and Kuschner made the interesting observation that the progress of human tuberculosis inoculated into guinea pigs was not retarded by usnic acid if used alone but that usnic acid augmented the effect of streptomycin. They used five groups of guinea pigs which had been inoculated with tubercle bacilli and treated as follows: I. Untreated; II. Usnic acid only (20 mg of usnic acid in oil-Tween 80 mixture subcutaneously every day for 6 days, then 10 mg daily for 24 days); III. High doses of streptomycin (6 mg daily); IV. Low doses of streptomycin (2 mg daily); V. Usnic acid plus low doses of streptomycin. Seventy guinea pigs infected with tuberculosis were compared in the various groups. Usnic acid alone did not effect any retardation of the disease nor did low doses of streptomycin (2 mg daily). A comparable, marked retardation in the development of the disease was obtained by each of two modes of treatment: (a) with high doses of streptomycin, and (b) with usnic acid combined with low doses of streptomycin.

In 1948, one of the present writers and his co-workers carried out animal experiments to obtain data on the toxicity and effect on tuberculosis of l-usnic acid isolated from reindeer moss. A series of 23 guinea pigs, ranging in weight from 290 to 900 g, were used as test animals, 7 of the animals serving as controls. An intraperitoneal inoculation of 1/1000 mg of a pure culture of *Myc. tub. hominis* was made into 16 guinea pigs. Eight animals in this group and 4 animals in the control group received *per os* a daily dose of 15 mg of sodium usneate, the sodium salt of l-usnic acid. At the end of 8 weeks the animals were killed and weighed, and macroscopic and microscopic examinations were made. No noteworthy differences were found in regard to the changes in weight or in the macroscopic appearance of the treated and untreated groups of tuberculous guinea pigs, and no toxic symptoms were observed. On the other hand, the microscopic examination of the spleen,

liver, lungs and lymph nodes revealed a more benign type of tuberculosis and a lesser involvement in most of the treated tuberculous animals. This was most clearly indicated by the finding that caseous necroses were rarely present in the group of treated mice.

The same workers published in 1950 the results of further experiments on tuberculous guinea pigs. A series of 36 test animals was employed. The animals were weighed and a part of them were infected with human tubercle bacilli of 3 different strains (967, 968 and IV). The test animal groups and the dosages of l-usnic acid are shown in the following table.

Dose of l-usnic Acid	No. of Guinea Pigs Inoculated with Tbc Strain No.			Uninfected Guinea Pigs	Total
	967	968	IV		
No treatment	4	4	5	3	16
15 mg orally	4	4	2	5	15
50 mg orally	—	—	2	—	2
5 mg subcutaneously	—	—	2	1	3
Total	8	8	11	9	36

The guinea pigs were sacrificed at the end of 8 weeks. At macroscopic examination it was again observed that there were no noteworthy differences in the body weight of the animals in the different groups. Attention was paid especially to the macroscopic appearance of the spleen, lungs, lymph nodes and liver and to the weight of the spleen. Marked differences were seen especially in the weight of the spleen in the different groups. The average weight of this organ in the uninfected guinea pigs, whether treated or not treated with usnic acid, was 770 mg, whereas that of infected animals which had not received usnic acid averaged 1,700 mg. The average spleen weight of the tuberculous guinea pigs was thus more than twice as high as that of the healthy animals. On the other hand, the average spleen weight in the group of tuberculous guinea pigs treated with usnic acid was 1,111 mg, *i.e.*, lower than that in the infected groups not treated with usnic acid but nevertheless fairly high. The tuberculosis in the guinea pigs infected with strain IV was macroscopically more severe than that induced with strain 967.

The microscopic examination revealed no noteworthy differences between the different test groups. A comparison of the results

of microscopic and macroscopic examinations of the animals infected with tbc strain 967 (isolated from a benign, productive case) and strain 968 (from a productive-exudative case) showed no difference between the group treated with usnic acid and that not treated with this substance. A clear difference was seen in this respect in the groups infected with tbc strain IV (isolated from an exudative malignant case), for the incidence of caseous necrosis in the untreated group was twice as high as in the treated group.

A peroral dose of 15 mg of l-usnic acid was found more beneficial than a dose of 50 mg. The two test animals that received subcutaneous injections of usnic acid showed marked tuberculous changes. It was found that: (1) Guinea pigs treated with l-usnic acid differed to some extent macroscopically from untreated guinea pigs, this being most evident in the weight and appearance of the spleen; (2) In the group receiving l-usnic acid the malignant cases also showed microscopically a positive tendency to calcification and an increased amount of connective tissue; (3) The incidence of necrosis was somewhat lower in the animals treated with l-usnic acid; (4) The differences between guinea pigs treated and not treated with l-usnic acid were most clearly seen in the groups infected with a tbc strain isolated from an exudative case. In animals inoculated with strains isolated from productive cases the changes were not as distinct.

Jäntti published in 1952 the results of his experiments on guinea pigs in which pure usnic acid isolated by Meretoja was used. He injected subcutaneously into 15 guinea pigs 1 ml of a 7 days old growth of *Myc. tuberculosis* in Dubos' fluid medium. The bacterium had been shortly before isolated from a tuberculous human patient. A series of 14 animals was given simultaneously 0.1 gm of usnic acid. A week later 13 of these animals received a similar dose, in the following week 12 animals, and so on. The number of treated animals thus decreased by 1 each week. This was continued for a period of 9 weeks, at the end of which all the animals were sacrificed. One test animal had died during the fourth week. The most marked tuberculous changes were observed in two guinea pigs. One of these had been given no usnic acid and the other had received it twice. The mildest forms of disease were noted in the guinea pigs injected with usnic acid during at least 5 weeks; one-half of this group had been treated during the full period of the experiments.

OBSERVATIONS ON THE THERAPEUTIC EFFECT OF L-USNIC ACID

Already in 1898 Chiba observed that tinctures prepared from several species of *Usnea* were beneficial in the treatment of *lymphadenitis tuberculosa colli*.

In 1949 one of the present authors published a case of *lupus vulgaris* which had been treated with a solution and ointment of usnic acid. This was a female patient 34 years of age, with cicatriza-

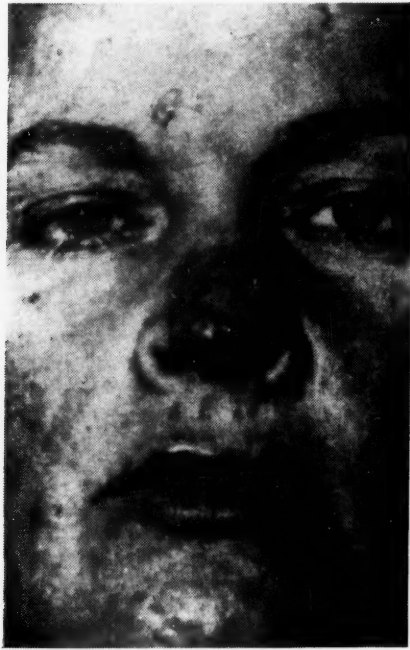


Fig. 3.

tions in the lungs observed in 1941. Two years later tuberculous glands were found on her throat. In the same year a thick-crusted eczema developed on the left side of her nose, spreading into the nostrils. Simultaneously there was pain in the palate. The patient's tuberculosis had not called for treatment for some years. The eczema had resisted all treatment. When usnic acid treatment was started in 1949 the skin on the patient's nose was red, the nose was swollen and the lips were bleeding. The left side of the nose was partly destroyed and there were suppurative wounds (fig. 3). In the anterior

portion of both nares there was dry crust. The mucous lining on the anterior walls of the nasal septum was rough and bleeding, elsewhere the mucosa was hard. The uvula was destroyed. The larynx was normal. Histopathologically this was a typical case of *lupus vulgaris*.

Local treatment of the eczema with usnic acid was started by spreading 1 per cent usnic acid acetone on the affected parts thrice daily during one week. During the second week warm carbowax ointment containing 1 per cent usnic acid was applied, followed, after it began to be absorbed, by 1 per cent *unquentum molle*. This treatment was also carried out thrice daily. After 2 weeks' treatment the swelling began to subside and the lesions on the upper lip were reduced in thickness. Four weeks after commencement of the treatment the patient's skin was smooth, softer but blotchy, and the swelling had disappeared. The patient then continued treatment at home and two months later her nose was healed to a remarkable degree. The skin was still reddish but fairly smooth, there was hardly any crust formation in the nares, and the roughness on the nasal septum had disappeared. The external lesions were still present on the nose. The pharynx and larynx were normal.

Since publication of this case in 1949 it has been followed up during 4 years. On April 13, 1950, active processes were no longer found. In the anterior part of the septum, where there earlier had been granulations, only a smooth-edged pinhead-sized perforation was now seen (fig. 4). On January 10, 1951 the left nasal wall showed chiefly scar formation. Almost at the site of the scars there was a slightly reddish area where the mucous lining was absent. A biopsy was taken from this area (chronic inflammation). The septal perforation was unchanged. In 1952 a skin graft was made. Examination of the nose and pharynx on January 19, 1953 showed in the septum a smooth-edged perforation about 4 mm in diameter. The nasal passages were fairly wide. The left septa were in a good condition. There was a small amount of crust in the nasal passages, and at about the middle of the lower right septum there was a small white nodule which did not bleed when touched. The biopsy revealed a chronic inflammation. The condition of the epipharynx was good, the soft palate was scarred, and the uvula absent. (Fig. 5).

In 1951 Kalima and one of the writers discussed the use of usnic acid in surgical tuberculosis and reported favourable results in a

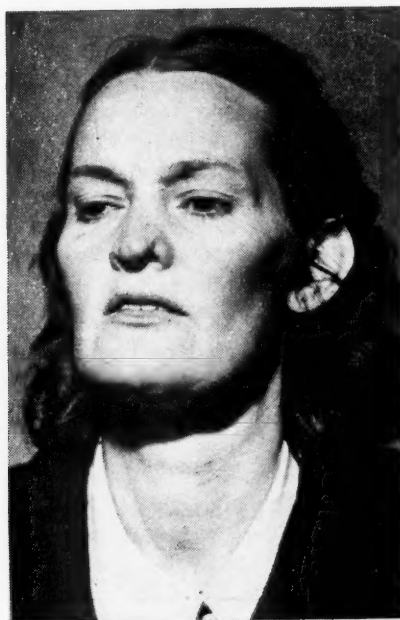


Fig. 4.



Fig. 5.

case of cold abscess and two cases of spondylitis. They had also tried usnic acid in bilateral renal tuberculosis and had obtained good results in one case each of pulmonary tuberculosis and of indefinite premenstrual disturbance. Observations were also described in the dermal application of usnic acid in the form of ointment, and an interim report was given of the above case of *lupus vulgaris*. A mixture of usnic acid and thyrotricin was also reported to have proved beneficial. On the other hand, visible benefit was usually not obtained in a short time in chronic cases of tuberculosis of the skin.

It is now possible also to give a report on 9 cases in which treatment with usnic acid was started in 1948 or 1949, since a sufficiently long period of control has now elapsed.

CASE REPORTS

Case 1. — The patient was a 31-year-old automobile driver. A diagnosis of pulmonary tuberculosis had been made in 1943 and thoracoplasty had been performed in 1946. Since June 1947 there had been abdominal pain and diarrhea.

On admission to the hospital on May 21, 1948 he was in a poor general condition and weighed 61.3 kg. Body temperature was 37.4° C and SR 14 mm/1 hr. Tubercle bacilli were present in the sputum. X-ray examination of the thorax revealed a post-thoracoplastic condition on the left side. In the right middle and lower fields there were fine linear shadows. Dg: 1) *Tub. pulm. II l. a. exs. prod.* 2) *St. post thoracoplastiam l. sin.* 3) *Tub. ileocecalis (Irrigoscopy).*

Usnic Acid Treatment. — Powdered sodium usninium was administered orally during a period of 3 months in daily doses of at first 0.4 gm and later 0.5 gm. The patient's general condition improved; for instance, weight increased by 3 kg and body temperature receded to normal. The abdominal pain disappeared, although no relief had previously been obtained from the usual symptomatic medical treatment (opium and belladonna). His condition improved to such a extent that he desired to be transferred home. Two months later he was rehospitalised because of a relapse of the gastric pain and subfebrile temperature. The pain was once more allayed by sodium usninium treatment, at first in a dose of 0.5 gm and later of 1 gm daily. Because of the persistent subfebrile condition he received 10 gm of PAS daily for 1 month but without benefit. The elevated temperature was believed to be due to a fistula in the thoracoplasty scar, and following surgical repair of this lesion the patient gradually became afebrile. Two months after operation the abdominal symptoms returned, but disappeared after 2 months' treatment with PAS, which was discontinued on November 9, 1949. — In the following spring the patient's condition again became aggravated but administration of sodium usninium for 2 months, now in

a daily dose of 2 gm, removed both the abdominal pain and the vomiting, and the feces became firm. In irrigoscopy the tuberculous changes were now slight and circumscribed. On May 28, 1950 the patient was dismissed for continued treatment at home. At *follow-up examination* in January 1954 he was in a moderately good condition. There was occasional abdominal pain but no diarrhea and he had been able to perform light work.

Case 2. — This was also an automobile driver, 66 yrs. of age. A diagnosis of pulmonary tuberculosis had been made in 1946 and recently there had been abdominal pain. On hospitalisation on February 18, 1949, the patient's physical condition was poor, weight 48 kg, feces bloodstained, SR 75 mm/1 hr., and there was a finding of tubercle bacilli in the sputum. Laryngoscopic examination showed swelling of the epiglottis and a deep suppurative lesion on its margin. Roentgenologically both lung fields were almost entirely covered with spot shadows and there were cavities in the upper fields. Diagnosis: 1) *Tub. pulm. III l.a. exs. cav.*; 2) *Perichondritis epiglottidis tuberculosa*; 3) *Suspicio tub. intestini*.

Usnic Acid Treatment. — The peroral administration of powdered sodium usnicum in a dose of 0.5 gm twice daily was started immediately and continued for 2 months. The temperature regained the normal level, the abdominal pain disappeared and the feces became firm. At first the sedimentation rate dropped clearly but increased again towards the end of the treatment. The laryngeal and pulmonary processes remained practically unchanged. The abdominal symptoms recurred almost immediately after discontinuation of medical treatment. The sodium usnicum therapy was resumed for nearly 1½ months until June 1, 1949. During this time the gastric symptoms were absent and the body temperature normal. On the other hand, no effect was seen on the laryngeal and pulmonary conditions. The patient died later on September 25, 1949.

Case 3. — In this patient, a 50-year-old carpenter, pulmonary tuberculosis had been recognised in 1946. On admission to the hospital on February 10, 1949 his physical condition was good. Weight was 62 kg, body temperature 37.2° C and SR 40 mm/1 hr. Tubercle bacilli had been found in the sputum. In the roentgenogram there were close small spots over the right upper and centre fields. Dg: *Tub. pulm. II l.dx. exs. prod.*

Usnic Acid Treatment. — The patient received 1 gm of sodium usnicum twice daily for 2 months. During this treatment he was practically afebrile. The sedimentation rate declined at first but increased again towards the end of the treatment. Leukocytosis and a shift to the left disappeared from the white cell count and the hemoglobin level increased significantly. Shadows in the roentgenogram had clearly diminished and were more band-shaped, but after discontinuation of the medical treatment they again increased. PAS was therefore administered for 2½ months (14 gm daily), followed by streptomycin (0.5 gm daily) for 1 month up to October 7, 1949. The temperature continued afebrile but roentgenologically there was no improvement in the condition. The patient was dismissed for home treatment, his condition gradually became aggravated and the case had a fatal termination on September 29, 1951.

Case 4.—A painter, 37 yrs. of age, had a recognised history of pulmonary tuberculosis since 1947. He was in a poor condition on hospitalisation on November 9, 1949, with a weight of 62 kg, body temperature 38.5° C and SR 65 mm/1 hr. There were no gastric symptoms. X-ray examination showed over both lung fields close shadows which diminished downwards. Dg: *Tub. pulm. III l.a. exs.*

Usnic Acid Treatment.—Sodium usnicum was administered for 1½ months, at first 0.5 gm twice daily and towards the end 1 gm twice daily, followed by PAS therapy during 1 month. Neither treatment was of avail and death ensued 2 weeks later.

Case 5.—In this patient, a 35-year-old mechanic, pulmonary tuberculosis had been diagnosed in 1943. There had been abdominal pain and diarrhea since early 1948. On admission on October 22, 1948 his condition was poor, body temperature 37.9° C, body weight 34 kg, SR 91 mm/1 hr, and the feces were liquid. Roentgenologically there was a good pneumothorax on the left; the shadows in the parenchyma of both lungs appeared to be distinctly circumscribed. Dg: 1) *Tub. pulm. II l.a. exs. prod.*; 2) *Suspicio tub. intestini.*

Usnic Acid Treatment.—The patient received sodium usnicum for 1½ months. The dosage was at first 1 gm three times daily but since it produced slight nausea and palpitation of the heart it was reduced to 0.5 gm given twice daily. During the treatment the abdominal pain and diarrhea decreased and the feces became firmer. The hemoglobin concentration increased clearly but the sedimentation rate and temperature were practically unchanged. PAS was now administered (daily dose 10 gm) for 1 month. The improvement in the patient's general condition continued but the subfebrile temperature did not completely subside. He was dismissed in the beginning of 1949 in a moderately good condition for continued treatment at home. A *follow-up inquiry* revealed that the patient had died on July 6, 1952.

Case 6.—The patient was a 32-year-old labourer, with a diagnosis of pulmonary tuberculosis made in 1938. Since October 1946 there had been albuminuria. On admission to the hospital on September 10, 1949 he was in a poor physical condition, weighed 46 kg, and had a body temperature of 37.3° C. The sputum was tb positive, alb. 8‰, and SR 135 mm. In the roentgenogram there were fairly marked large spots and an apple-sized clarification over the upper middle field of the left lung. Diagnosis: 1) *Tub. pulm. III l. sin. exs. cav.* 2) *Amyloidosis?*

Usnic Acid Treatment.—Sodium usnicum was administered during 2 months in a dose of 1 gm twice daily. The patient's physical condition improved, as shown for instance by a gain of 4.5 kg in weight and an afebrile temperature. The volume of sputum also declined greatly. Medical treatment was continued with streptomycin, given in a dose of 0.5 gm twice daily for 1 month. There was continued improvement in the patient's condition but even yet no change was seen in the roentgenogram and the high sedimentation rate also persisted (93 mm). He was discharged on

November 28, 1950 for home care. *Follow-up inquiry:* The patient had died on January 9, 1952.

Case 7. — The patient, a labourer 35 years of age, was in a poor general condition and weighed 66 kg. The temperature was 37.0°, SR 75 mm/1 hr. There were no gastric symptoms. In the roentgenogram there were moderately dense shadows with diffuse margins over the upper left lung field and almost the entire right lung field. A cavity was seen under the right subclavicular fossa. Diagnosis: *Tub. pulm. III l.a. exs. cav.*

Usnic Acid Treatment. — A daily dose of 1 gm \times 2 of sodium usnicum was given for 2 months. Some improvement occurred in the patient's general condition, the SR declined to 5 mm/1 hr and the shift to the left disappeared from the blood picture. The volume of sputum was also notably diminished but no roentgenological changes were seen. The patient was dismissed for home care on April 14, 1949. He died on October 30, 1949.

Case 8. — A laboratory assistant 25 years of age had a history of pulmonary tuberculosis which had been diagnosed in 1940. On admission on November 28, 1948 she was in a good physical condition. Body temperature was 37.2° C, SR 16 mm/1 hr, and the roentgenogram showed fairly sparse linear and dotted shadows over both upper fields, however more on the right side. Dg: *Tub. pulm. II l.a. prod.*

Usnic Acid Treatment. — She received sodium usnicum 0.5 gr \times 2 daily for 2 months, during which the body temperature and the sedimentation rate receded to normal and the shift to the left disappeared from the blood picture. In the roentgenogram the shadows had decreased and become more sharply defined and linear. *Follow-up inquiry,* April 12, 1954: The patient had been steadily at work, there had been no fever, the shadows in the roentgenogram had remained circumscribed and no new foci were seen.

Case 9. — The patient, a 36-year-old office manager, had pulmonary tuberculosis which had been diagnosed in 1942. Suppurating fistulas had been present in the anal region for about 3 months. On hospitalisation on November 8, 1949 he was in a good physical condition, weighed 75 kg, had a normal body temperature of 36.8° C and SR 5 mm/1 hr. No tubercle bacilli were found in the sputum but they were present in the fistular pus. The roentgenogram showed a good pneumothorax on the right and linear shadows from the left hilus to the subclavicular fossa. In the anal region were two fistular openings, with a profuse discharge of pus. Dg: 1) *Tub. pulm. II l.a. prod.* 2) *Pneumothorax artificialis l.d.x.* 3) *Fistula ani tuberculosa.*

Usnic Acid Treatment. — A 5 per cent usnic acid ointment was injected into both fistulas during 3 weeks.

The discharge of pus stopped and the fistulas closed spontaneously. Two weeks later a new anal fistula appeared at a new site. Since fluid containing tubercle bacilli had simultaneously collected in the pneumothorax cavity and the temperature was subfebrile, streptomycin therapy was given for 5 weeks. The temperature receded to normal and the anal fistula healed 1 month after discontinuation of the medical treatment.

Follow-up inquiry, March 20, 1954: The patient was in a good condition and afebrile. The pneumothorax was discontinued. There had been no recurrence of anal fistulas.

In 6 of these 9 cases the serum inorganic phosphorus was determined according to Fiske and Subbarow and Lohmann and Jendrassik and the alkaline phosphatase according to Lehman's modification. The serum inorganic phosphorus increased or remained unchanged in 2 cases and dropped in 4 cases after 2 weeks' administration of usnic acid per os. The serum alkaline phosphatase increased or remained unchanged in 5 cases and declined in 1 case.

DISCUSSION

It has been demonstrated in numerous in vitro tests and animal experiments that usnic acid has a bacteriostatic action on tubercle bacilli. The clinical cases in which we used usnic acid therapy were mostly severe cases with a poor prognosis. This case series is small, it is true, but the follow-up periods were in our opinion sufficiently long. From the results obtained it appears probable that usnic acid has some effect on the course of human tuberculosis also. This action is seen chiefly as a decrease or complete disappearance of certain symptoms. Diarrhea and other gastric and intestinal symptoms of tuberculotics were primarily affected by usnic acid. The substance also had a beneficial effect on tuberculous anal fistula and seems to have completely healed the patient with lupus vulgaris. On the other hand, usnic acid was not seen to have any notable effect on tuberculous changes in the lungs in our series except in the mildest case of pulmonary tuberculosis.

On the basis of this series of patients it therefore appears that the therapeutic action of usnic acid on tuberculosis patients, when given in this form per os and frequently when administered cutaneously, is limited to an alleviation of the symptoms. However, the results of recent tests would indicate that by combining usnic acid with various other chemical compounds it probably will be possible to secure better therapeutic results, as has been demonstrated by Marshak with usnic acid and streptomycin in animal experiments and by Virtanen *et al.* with Conteben and sulphaguanidine in vitro.

SUMMARY

The authors have reviewed earlier experiments carried out by them and by other investigators on the tuberculostatic action of l-usnic acid *in vitro* and *in vivo*. They have also made a survey of the reports in the literature relating to the therapeutic value of l-usnic acid in human tuberculosis. To supplement their earlier findings on this point the authors administered l-usnic acid to 9 patients with tuberculosis, one of whom also had anal fistulas. The substance had the effect of decreasing the symptoms, especially diarrhea, and it healed the fistulas. Very good results were also obtained in the treatment of a patient with lupus vulgaris. Tuberculous changes in the lungs, on the other hand, were not affected by usnic acid to any noteworthy extent.

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